THE BIOLOGY OF *DIADASINA DISTINCTA* (HOLMBERG, 1903) (HYMENOPTERA: ANTHOPHORIDAE)

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Abstract.—*Diadasina distincta* Holmberg is a solitary, multivoltine, and philopatric anthophorid bee that nested in aggregations on a vegetation-free dirt road in direct sunlight. Nests were provisioned by each female, and there were no burrow connections. Despite that 63 different pollen types had been found in the nests, *D. distincta* is oligolectic preferring *Ludwigia suffruticosa* L. (Onagraceae). Mating was promiscuous with males strongly competing for mates. Adults appeared sensitive to low light intensity, lowering nesting and mating activities during cloudy days. Females remained in their burrows at night and males probably in the surrounding vegetation. Nests were heavily parasitized by an *Anthrax* sp. (Bombyliidae) and by *Leucospis genalis* Boucek (Leucospidae). Both bee and parasite prepupae of the previous generation remained dormant in the cells from October to February.

Key Words: *Diadasina distincta*, Anthophoridae, nesting behavior, life-cycle, parasitism, *Anthrax*, Bombyliidae

The biology and behavior of neotropical solitary and presocial bees are poorly known. In Brazil, there have been some studies on biocoenotic structure (Sakagami et al. 1967, Laroca et al. 1982), some aspects of nesting biology of several species (von Ihering 1905), habitat selection by gregarious nesters of earthen banks (Michener et al. 1958) and their nesting behavior (Michener and Lange 1957, 1958 a, b, c, d, e; Michener and Seabra 1959; Sakagami and Moure 1967; Sakagami et al. 1967). Except for the study on nest spatial distribution patterns (Martins and Figueira 1992) and the survey record in the state of Rio Grande do Sul (Wittman and Hoffman 1990), there are no studies on the biology and nesting behavior of *D. distincta*. We present here data on the biology and associate organisms of *D. distincta*.

MATERIAL AND METHODS

Observations were made at the Estação Ecológica do Campus da Universidade Federal de Minas Gerais (UFMG), Belo Horizonte, Minas Gerais, Brazil, from January 1991 to October 1992. The individual's daily and monthly activities at the aggregation were ascertained by observation two days per week from March to August 1991, totaling 180 h. In the following months the observations were done opportunistically in the higher activity period between 11:00 and 15:00 h, for a total of 150 h.

The average monthly temperature and total monthly rainfall records were from the Estação Climatológica do Ministério da Agricultura in Belo Horizonte.

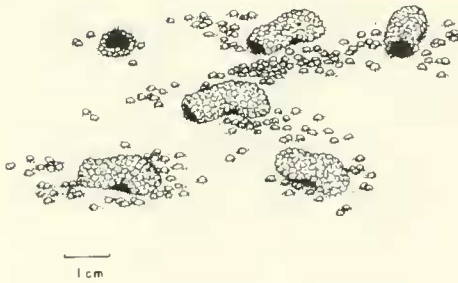Twenty nests were excavated to verify the existence of cell or burrow connections. Sol-

Fig. 1.   Turret morphology of *D. distincta.*

Fig. 2.   Nest structure of *D. distincta* showing vertical cell and nest entrance closures. (natural size, no turret).

ubility degree for water was determined submerging two cells in water for 10 min and two others for 24 h. The nest depths and turret sizes were measured with a caliper. We excavated three 20 cm² samples from which we obtained 186 cells that were measured and kept in vials at 25°C and 12 hours photoperiod until bee or parasite emergence. Averages and standard deviations were calculated for all the measurements.

In September, November, and January we excavated 10 nests each month to ascertain the developmental stages of both bees and parasites. We opened 100 cells in the laboratory to analyze their contents, and observed the post-emergence behavior of 10 males and 20 females of *D. distincta.* Voucher specimens of *D. distincta* are deposited in the Snow Hall Museum of Entomology of the University of Kansas and in the Laboratório de Ecologia e Comportamento de Insetos do Depto. de Biologia Geral da Universidade Federal de Minas Gerais (UFMG), in which are also deposited voucher specimens of associated insects.

RESULTS

Nesting behavior

The nesting site was in a vegetation-free dirt road in direct sunlight. Females excavated their isolated nests in dry and compact sandy soil, softened with water brought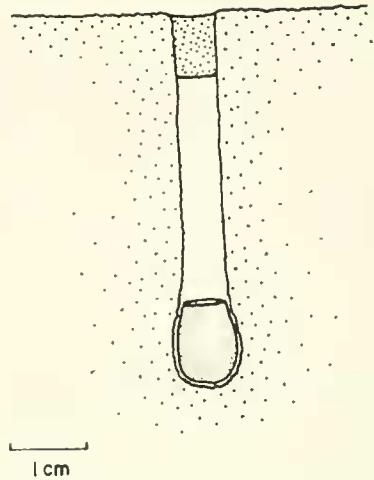 to the nests in their crops. The nests were excavated with the mandibles and the soil pushed out by hind legs and abdominal movements. The soil pellets were then pushed out by the hind legs and remained scattered around the nests entrances (Fig. 1). Each bee built also a curved turret at the nest entrance (Fig. 1). These turrets were frequently rebuilt when destroyed by rainfall or when bees were excavating or provisioning the burrow. Complete nests (n = 15) were 4.0 ± 0.3 cm vertical one-celled burrows with a 0.9 ± 0.2 cm in diameter (Fig. 2). Cells (n = 64) were 0.79 ± 0.07 cm in diameter and 1.05 ± 0.07 cm in length. We estimated that there were 12,400 nests in the 4000 cm² aggregation, based on the 186 cells we found in our sample of 60 cm². This aggregation has persisted for at least 5 successive years at the same site. H. R. Pimenta (pers. comm.) first recorded the aggregation in 1988, 1989, 1990. Burrow and cells were molded by the female pygidium which tamped the soil. Thus both burrow and cells walls become very smooth. The larvae lined the cell walls with a layer of fecal material overlapped by a thin cellophane-like layer of netlike silk threads that waterproofed the cells. Both cells sub-

mersed in water for 10 min and 24 hours showed no water infiltration in the larval chamber. Although the nests were located side by side and distributed in a regular fashion (Martins and Figueira 1992), there were mistakes by bees when they returned from provisioning trips, i.e. bees entered the wrong nests. However, shortly afterward, such bees returned to their own nests. Despite nest proximity there were no internest connections.

Nests were individually provisioned by females with pollen of 63 plant species. Nevertheless, only 5 types had frequencies higher than 5% in the samples. Among these types, *Ludwigia suffruticosa* L. (Onagraceae) had frequencies from 60% to 99%. Therefore, *D. distincta* is an oligolectic bee preferring *L. suffruticosa* (Martins and Borges, manuscript in preparation).

The pollen, carried on rigid hairs of the scopa of hind legs, was molded into a ball in each cell, then an egg was laid beneath it on the floor of the cell. Eggs measured 1.86 ± 0.14 mm (n = 3) in length. Bees filled only one fourth of the burrow before closing nest entrances with moist soil (Fig. 2). Sometimes, when turrets had not been destroyed by winds or rainfall, females closed the nests keeping the turret intact. Some females were observed initiating another nest shortly after completion of previous nests. The prepupae of *D. distincta* are flaccid, the larvae do not spin cocoons, and the cells are very strong.

The egg-adult development time observed in the field was 27.29 ± 2.24 days (n = 25), and there were at least three overlapping generations between February and October in this philopatric species.

Male patrolling and mating

Females slept in nests and males probably slept on the surrounding vegetation on which they also rested between patrolling periods. The males patrolled the aggregation on sunny days between 8:00 and 15:00 h, the peak of activity being around 11:00 h (n = 15 days). They were very sensitive to low light intensity. The patrolling flights quickly declined and stopped on cloudy days, or when the shadow of the observer fell on the aggregation. Then the males flew to the vegetation, where they waited for bright sun.

Mating in *D. distincta* seems to be promiscuous. Several males strongly compete for females. Newly emerged females and females provisioning nests were equally pursued. Males mounted the female's back, gripped her neck with his forelegs and her body with his middle and hind legs. Copulation lasted 38.06 ± 2.97 seconds (n = 30).

Most of the copulations occurred at nest entrances, and once a female was gripped by a male, two to five males may try to dislodge the successful one. The group may roll on the ground like a ball. In the laboratory, newly emerged males and females opened their own cell closures with their mandibles, emerging head first. A minute later they initiated copulation.

Nesting dynamics and life-cycle

The adult bees emerged at the end of the rainy season, and the nesting activities occurred during February to October, the drier and colder months of the year (Figs. 3, 4). The reproductive peak was in June, and no adults could be found from October to February, when the bees and their synchronized parasite prepupae are dormant in the cells. This oligolectic bee's life-cycle seems to be related to pollen availability, because its reproductive period is synchronized with the flowering period of its preferred host plant *L. suffruticosa* (as suggested by pollen analysis of cell contents). This plant is very abundant in a swamp within 50 m of the nesting site.

Aggregation dynamics

The aggregation-growing dynamics are quite interesting. Because nests were quickly built in close proximity and regularly distributed (Martins and Figueira 1992), the
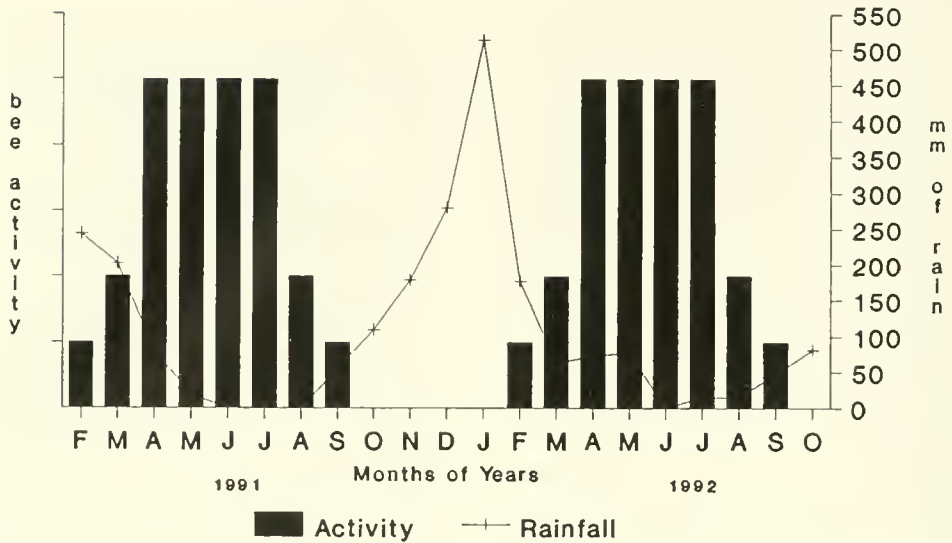
Fig.3. <u>D.</u> <u>distincta</u>'s activity level and rainfall variation at the Ecological Station of UFMG/BH/MG/BRAZIL.

Fig. 3. Activity level of *D. distincta* and rainfall variation at the Ecological Station of UFMG BH/MG/Brazil.

aggregation seemed to run in a "nesting wave," changing the direction when the nests were been constructed after rains or when it met vegetation. Around 400 m from the principal aggregation, there were 12 newly initiated sites, including from one to 200 nests, but these aggregations never grew like the principal one. In the 600 ha of the UFMG Campus only the principal aggregation persisted for several years. Patrolling males were less present in the incipient aggregations, and the primary parasite, *Anthrax* sp., was frequently found flying or perching near them.

Mortality factors and associate insects

Diadasina distincta was heavily parasitized by an *Anthrax* sp. (Bombyliidae). The rate of parasitism could be calculated because the Bombyliidae puparia remains in the burrow after the flies emerge. Because we were unable to distinguish among ag-

gregations of different size, it was impossible to relate the level of parasitism to aggregations of different sizes. However, in a somewhat isolated part of the aggregation, we observed that 25 out of 30 nests were parasitized by *Anthrax* sp. This evidence for the high intensity of parasitism is circumstancial.

The parasitizing behavior of *Anthrax* sp. is very similar to that of other bombyliids that parasitize solitary bees. Female flies patrolled the aggregation, hovered around nests, and twisted their abdomens to oviposit into the nest entrances. We do not know how the bombyliid larvae enter the bee cells. However, it seems like they would have to reach the cell before the bee securely closes the nest.

Leucospis genalis (Leucospidae) is another important parasite, but it seems to have a lower impact on the bee population than the bombyliid. The behavior of *L. genalis* was different from that of the bombyliid. Females flew around the nests, landing near
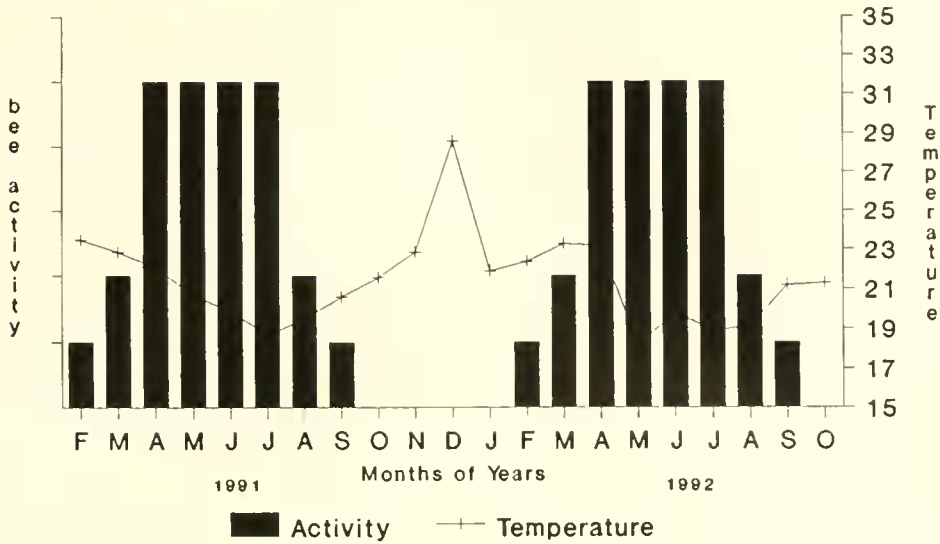
Fig.4. D. distincta's activity level and temperature variation at the Ecological Station of UFMG/BH/MG/BRAZIL.

Fig. 4. Activity level of *D. distincta* and temperature variation at the Ecological Station of UFMG/BH/ Brazil.

the turrets. Then they entered the nests through turret entrances while bees were away on provisioning trips. Sometimes a bee returned to the nest and met the parasite inside. Then it was aggressively pushed out by the bee. We observed two other bombyliid species rarely visiting the aggregation; however, we reared only *Anthrax* sp. and *L. genalis* in the laboratory.

Aggregation-associated ant lion larvae caused no detectable interference with the bees. Unusually heavy and persistent rains during January 1992 (513 mm) eroded the nesting site and exposed cells. Consequently, some *D. distincta* dormant prepupae and nest parasites were killed by desiccation or by patrolling ants such as *Solenopsis* sp.

An unidentified *Crematogaster* sp. invaded some nests (n = 5) and probably killed the bee or parasite larvae. A predator *Apiomerus* sp. (Reduviidae) was observed picking up a female at the nest entrance (H. R. Pimenta, pers. comm.). Termites built their subterranean nests below the aggre-

gation, but we do not know the kind and degree of interference they had on the aggregation dynamics. Occasionally the nests (n = 4) of another anthophorid bee, *Ptilothrix plumata* Sm., were taken over by *D. distincta*.

Trypoxylum aureovestitum Taschenberg (Sphecidae) was commonly seen flying and entering into the nests of *D. distincta*. An individual of *T. aureovestitum* entered many times one provisioning nest, picking up pollen with the mandibles scattering it around the nest. We excavated a nest with entrance closed with mud quite different from *D. distincta*'s closures. Inside we found two spiders and an egg. Thus the evidence is strong that *T. aureovestitum* can use *D. distincta* nests. However, we do not know its impact on the nesting dynamics of *D. distincta*.

A leaf-cutting bee *Megachile neoxanthoptera* Cockerell female built a cell inside a *D. distincta* burrow. However, this is only a general association between them because this leaf-cutter bee also uses other Antho-

phoridae and termite burrows, as well crevices in the soil, for nesting.

Discussion and Conclusions

Diadasina Moure has been placed as a subgenus of *Diadasia* (Michener 1979). Specimens we sent to Michener were identified as *Diadasina distincta* by A. Roig-Alsina. Thus Roig-Alsina and Michener believe that *Diadasina* is a valid genus. However, we found only reference to *Diadasia* in the Roubik's (1989) book about tropical bees.

Because there are no available data on the biology of *Diadasina* species, except for the records of *D. distincta*, *D. riparia* (Ducke) and *Diadasina* sp. in the state of Rio Grande do Sul (Wittmann and Hoffman 1990) and on the spatial distribution of nests (Martins and Figueira 1992), it is useful to compare the biology of *D. distincta* with related species of *Diadasia* for which there are some data.

Nests in *D. distincta*, as in most Emphorini (C. D. Michener, pers. comm.) are usually one-celled, and the burrows are shorter than those of *Diadasia* species. Species of the latter build linear series of cells in burrows branching from the terminus of the main burrow (Stephen et al. 1969).

There is high variability in the shape of the turrets among species of *Diadasia* (Stephen et al. 1969). The turrets of *D. distincta* differ in details from those of *Diadasia* species, which are vertically or horizontally straight in *D. olivacea* Cockerell and *D. enavata* (Cresson), compared with smoothly curved in *D. distincta*. Like *D. distincta*, among the species of *Diadasia* only *D. consociata* Timberlake does not build a tumulus around the turrets (Stephen et al. 1969). Like in other ground-nesting bees (Stephen et al. 1969), a secreted thread-like material is used to waterproof cells. Most Emphorini larvae on maturity line their cells with a layer of fecal material and spin a very thin cocoon (C. D. Michener, pers. comm.). Despite that *D. distincta* females lined their cells with fecal material, the larvae do not spin cocoons.

The rebuilding of the turret after damage is variable among solitary bees (Stephen et al. 1969). While building the burrow and provisioning the nest *D. distincta* always rebuilds the turret after any kind of damage. However, like other authors (see Stephen et al. 1969), we do not know the turret's function. The turret did not prevent intense parasitism by *Anthrax* sp. in *D. distincta*, nor parasitism by *L. genalis* (first record for *Diadasina*) whose females enter the nests throughout turrets. Otherwise, *Diadasia bituberculata* (Cresson) is heavily parasitized by the bombyliid *Villa* sp. (Linsley 1958) and there is no record of *Leucospis* parasitizing species of *Diadasia* (see Stephen et al. 1969, Roubik 1989).

Apiomerus reduviids are well-known predators of solitary bees on flowers (Linsley 1958, Roubik 1989). Predation at nest entrances has also been observed (Roubik 1989). Linsley (1958) observed mutillids parasitizing species of *Diadasia*. Although several species were present at the study site we did not observe parasitizing nests.

Because no incipient aggregation had been enlarged and males rarely patrolled them, we supposed that the aggregation formation and persistence could be dependent on the frequency of patrolling males. In spite males do not patrol aggressively the aggregation, the presence of a number of males flying on the aggregation could prevent flies from approaching the provisioning nest; thus reducing parasitism success (see Martins and Figueira 1992). From this hypothesis we would expect to find an inversely-density dependent parasitism. However, more data are called for in our attempt to understand the parasite functional response.

Males of some bees wait to mate with females on individual flowers. One example of this for *Diadasia australis* (Cresson) on cactus flowers was recorded by R. Brooks (Eickwort and Ginsberg 1980).

As in other bee species (Eickwort and

Ginsberg 1980), in *D. distincta* the lack of male aggressivity in patrolling the aggregations allow other males to do the same. As in other bees, such as *Diadasia rinconis* Cockerell (Ordway 1987), when a receptive female of *D. distincta* is encountered by one male, other males will also attempt to copulate with her, resulting in a tumbling mass of males surrounding the female. However, copulation in *D. rinconis* lasts several minutes, whereas in *D. distincta* it lasts only a few seconds.

The life history and nesting biology of *D. distincta* is similar in some respects to *Diadasia rinconis* that nests in Arizona and Texas (Ordway 1987). Both species nest in dense aggregations. The egg-adult period lasts about 20 days in *D. rinconis* and 27 days in *D. distincta*. Adults emerge from the ground and mate from 09:00 h to noon in the same dry months of the year (see Figs. 3 and 4 in this study and fig. 2 in Ordway 1987). But, ". . . it is unlikely that females of *D. rinconis* could nest in hard dry soil in late May and June because newly constructed cells were consistently found in moist soil (Ordway 1987)." Conversely, females of *D. distincta* nested actively in the hard dry soil because they softened it with water gathered in their crops. Moreover, some *D. rinconis* pupae and adults apparently remained dormant in their cells until the next year, similar to the prepupae of previous generation of *D. distincta*, and in the univoltine *Diadasia opuntia* Cockerell (Ordway 1987). These species became dormant in different stages of development by using the same strategy to circumvent unfavorable weather or food scarcity period. Nevertheless, it is more likely that food scarcity is the primary factor preventing all year reproduction in *D. distincta* because it is an oligoletic species.

Except for *Diadasia afflicta* (Cresson), which is bivoltine, *D. rinconis* and other known species of *Diadasia* are univoltine. Although *D. distincta* was found to be multivoltine, its reproduction is seasonally con-

strained, probably by lack of preferred food (Martins and Borges, manuscript in preparation). Considering that both species could be exposed to similar selective pressures and that the reproduction in *D. distincta* is seasonal, it is unknown why *Diadasia* species evolved univoltinism and *D. distincta* multivoltinism. Lowering of the egg-adult time of development *Diadasia* spp. would select for multivoltism. Using the same reasoning, Matthews (1991) asked why among temperate-zone sphecid wasps the most widespread condition is univoltinism, since there is ample time for the development of a second and even a third generation.

The evidence for dormancy in the tropical Hymenoptera is very scant (Denlinger 1986). The only case cited by Denlinger (1986) in the Aculeata is for the anthophorid *Epicharis zonata* Lepetelier. We suspect that dormancy in the tropical Hymenoptera is more common than previously supposed because we have evidence for prepupal dormancy in three sphecid and two other solitary ground nesting bees (Martins 1993, Martins and Almeida, manuscript in preparation).

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